Annals of Clinical and Analytical Medicine
Original Research

A histological study for the evaluation of potential harmful effects of orally ingested iron oxide nanoparticles in mice

Mubin Mustafa Kiyani[1], Syed Ali Imran Bokhari[1], Asmara Syed[2], Hamza Rehman[1], Ekramy Elmorsy[2-3], Syed Sajid Hussain Shah[2]
[1]Department of Biological Sciences. International Islamic University, Islamabad, Pakistan
[2]Department of Pathology, Faculty of Medicine, Northern Border University, Arar, Kingdom of Saudi Arabia
[3]Department of Clinical Toxicology, Faculty of Medicine, Mansoura University, Egypt

Abstract

Aim: The aim of this study is to search for the possible adverse effects of orally ingested iron oxide nanoparticles on the liver, kidney, and skeletal muscle of BALB /c mice.

Materials and Methods: Iron oxide nanoparticles were prepared with the co-precipitation method and characterized using Scanning electron microscope (SEM) and Energy-dispersive spectroscopy (EDS). BALB /c mice were chosen as an experimental animal model. Twenty-four mice were classified into four groups with six mice in each group. Group 1 was named control group. The second, third, and fourth groups were experimental groups which were given iron oxide nanoparticles daily in concentrations of 125, 250, and 500 mg/kg bodyweight for 21 days via oral ingestion. One day after the last feed of nanoparticles, the tissue specimen from the liver, kidney, and skeletal muscle were taken and processed for histopathological evaluation to study the evidence of any toxic effects of iron oxide nanoparticles on these organs.

Results: The histopathological assessment of the liver, kidney and skeletal muscle tissue of the three experimental groups revealed no evidence of necrosis, inflammation, or any degenerative changes in comparison to the control group samples.

Discussion: Oral ingestion of spherical shaped iron oxide nanoparticles (average diameter 50nm) is safe and there was no histological evidence of any toxic effects on the liver, kidney, and skeletal muscle of BALB /c mice in concentrations up to 500 mg/kg body weight for 21 days. These findings are in accordance with some studies but certain studies reported the toxic effects of iron oxide nanoparticles. This means that the size, shape, and route of exposure to iron oxide nanoparticles can affect their extent of toxicities.

Keywords

Iron oxide; Nanoparticles; Toxic effects; Histopathology

DOI: 10.4328/ACAM.20244 Received: 2020-06-10 Accepted: 2020-07-11 Published Online: 2020-07-20 Printed: 2021-03-01 Ann Clin Anal Med 2021;12(3):238-241
Corresponding Author: Syed Sajid Hussain Shah, Faculty of Medicine, Northern Border University, Arar, Saudi Arabia.
E-mail: prof.sajid99@gmail.com P: 0096653775964
Corresponding Author ORCID ID: https://orcid.org/0000-0003-3425-6293

Introduction

In the present era, nanotechnology is an emerging important tool in many fields such as biomedical, health care, food and agriculture, environmental control and protection, energy, construction, automobile industry, textile industry, electronic, defense and aerospace. The nanoparticles are classified into different categories on the basis of their characteristics, sizes, and shapes such as carbon-based nanoparticles, metal nanoparticles, ceramic nanoparticles, semiconductor nanoparticles, polymeric nanoparticles, lipid-based nanoparticles [1].

The different types of metal nanoparticles such as silver, gold, iron, copper, cadmium, and platinum are being employed for various uses in the medicine (for diagnostic and therapeutic purposes) and in other sectors such as fruit preservation and water quality monitoring [2]. The unique characteristics such as superparamagnetism, large surface area, and less difficult separation methodology of the iron oxide nanoparticles make these nanoparticles more attractive for consideration of its application [3].

After aluminium, iron is the next most abundant metal present in the crust of the earth but despite the abundance of iron, a huge number of people from all over the world suffer from iron deficiency anemia. Anemia is quite common in children and pregnant women [4-7].

Optimum availability of oxygen is vital for the normal functioning of living cells while in case of iron deficiency anemia, the oxygen-carrying capacity of the erythrocytes reduces and has a negative impact on the cellular functions.

The use of iron oxide nanoparticles revealed positive results for the management of iron deficiency anemia [8].

With the rising trend in the use of metal nanoparticles like iron oxide, it would be very important to evaluate the possibility of adverse effects of these nanoparticles. The assessment of the impact of different doses and sizes of nanoparticles on the living tissue is very vital. The aim of the present research project is to find out any histopathological evidence regarding the toxic effects of iron oxide nanoparticles on the liver, kidney, and muscle tissue of mice.

Material and Methods

Synthesis and Characterization of FeO NPs

For the purpose of making average and uniform nanoparticles, a co-precipitation method was utilized, in which a 1:2 ratio of ferrous and ferric chloride were mixed together in 50 ml of de-ionized water. In this prepared mixture, an 8.5 M sodium hydroxide solution was added very slowly till the pH of the solution reached 11. The obtained mixture was washed many times using distilled water and was later on dried using an oven at 100 °C [9]. These particles were characterized using a Scanning electron microscope (SEM) and Energy-dispersive spectroscopy (EDS).

Treatment of animal groups

This research project was performed after getting ethical approval from the International Islamic university ethical committee. Four groups (1, 2, 3 and 4) of the BALB/c mice were made up of six animals in each group. The mice included in the control Group (1) were fed with a diet without iron oxide nanoparticles while the mice of Groups 2, 3, and 4 were fed for 21 days with the diet containing iron oxide nanoparticles 125, 250, and 500 mg/kg body weight of iron oxide nanoparticles, respectively.

Histopathological examination of specimens

The tissue specimens from the liver, kidney, and skeletal muscle of all groups of animals have been acquired and histopathologically examined for any morphological evidence of toxicity. The 10% buffered formalin was used as a fixative. The tissue processing was done by an automated tissue processor. The processed tissue was embedded in paraffin wax for the preparation of tissue blocks. The 3- 4 micron thick sections have been cut from the tissue blocks. The slides were stained with hematoxylin and eosin stain. The stained slides were examined by two histopathologists.

The liver specimens were assessed for any structural changes in the liver architecture and for the presence of inflammation or hepatocyte necrosis, apoptosis, and fibrosis. The specimens from the kidney were examined for any morphological alteration in the glomeruli, tubules, interstitium, and blood vessels. The muscle tissue was examined for any inflammation, necrosis, degenerative and regenerative changes.

Results

Characterization of synthesized iron oxide nanoparticles:

Synthesized FeO nanoparticles were subjected to SEM to evaluate the structure and size of the particles. It was seen that these iron particles were of spherical shape and had an average diameter of 50 nm (figure1A). From EDS analysis it was observed that the synthesized particles constituted of an adequate amount of iron and oxygen while trace quantities of sodium and chloride were also present (Figure 1B).

Histopathological evaluation:

Microscopic examination of liver from all experimental groups of mice revealed intact architecture of the liver without any evidence of hepatocyte balloon degeneration, necrosis, apoptosis, inflammation, or fibrosis. The slides made from kidney tissue of mice of all experimental groups revealed no histopathological evidence of any toxic effects on glomeruli, tubules, interstitium, and blood vessels. There is no evidence of any morphological abnormality in the muscle tissue specimens of all experimental groups.

Discussion

Nano-science is a rapidly developing field of science with promising results for the prosperity and well-being of humanity. Many substances are being used for the synthesis of nanoparticles and these nanoparticles are being evaluated for the potential beneficial effects. The studies reported positive results regarding the use of nanoparticles for the treatment of bacterial and viral infections [10-12].

The potential use of nanoparticles in the diagnostics and the therapeutics is being extensively studied, particularly the utilization of nanoparticles for the efficient delivery of drug to the targeted site without much exposure of normal cells to the toxic medicinal product [13]. The use of iron oxide nanoparticles based nanocarriers of anticancer drugs revealed less toxic effects of drugs on the normal cells [14, 15]. In the near future,

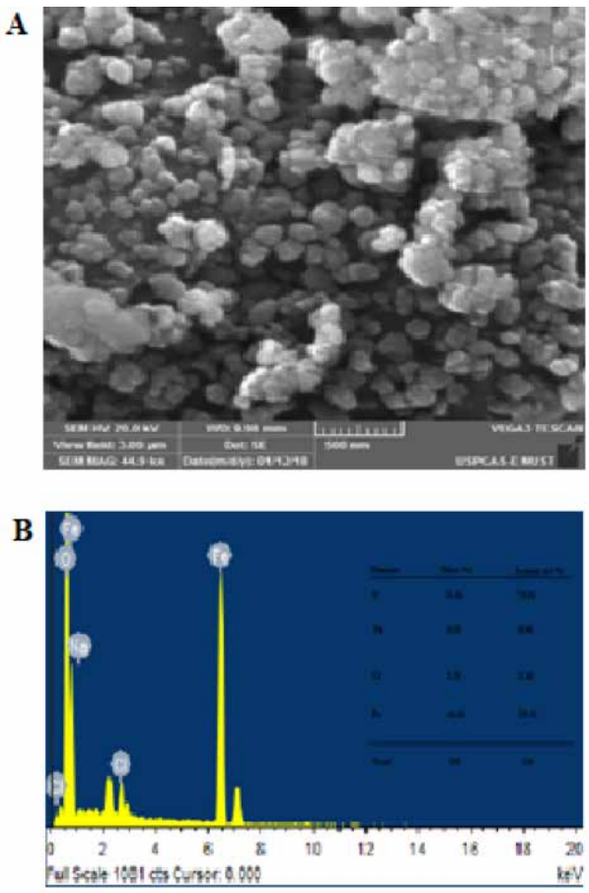

Figure 1. A: Scanning electron microscopy (SEM) imaging for the synthesised FeO NPs. SEM shows that these iron particles were of spherical shape and had an average diameter of 50 nm. **B:** Energy-dispersive spectroscopy (EDS) spectrum of synthesized FeO NPs. EDS shows that the synthesized particles constituted of adequate amount of iron and oxygen and trace quantities of sodium and chloride were also seen.

the use of nanoparticles for diagnosis as well as therapeutics is expected to rise. In this situation, the possibility of toxic effects associated with these nanoparticles may need to be studied extensively.

Oral ingestion, inhalation, and skin are the common portals of entry of nanoparticles in the human being. After absorption in the blood, these nanoparticles may accumulate in the liver and hepatocyte may be at more risk to bear the toxic effects of nanoparticles [16]. After degradation, the waste products are removed from the blood by the filtration process of the renal tissue. The kidney plays a vital role in the elimination of nanoparticles from the body [17]. After the liver, the renal tissue may also be exposed to the toxic effects of the nanoparticles.

In our study, the three groups of mice were exposed to 250 mg/kg body weight, 375 mg/kg body weight, and 500 mg/kg body weight of iron oxide nanoparticles respectively. The specimens from the liver, kidney, and muscle tissue from all experimental groups and control group were examined microscopically for any histological evidence of toxic effects. There was no histological evidence of untoward effects of iron oxide nanoparticles on the liver, kidney, and muscle tissue of mice in any experimental group. Some other studies also reported a similar finding in which they reported no evidence of toxicity associated with iron oxide nanoparticle [18, 19]. However, the toxic effects of iron oxide nanoparticles have been documented by certain studies [20-23]. The size and shape of iron oxide nanoparticles can modulate their toxic effects. As compared to sphere-shaped, the rod-shaped iron oxide nanoparticles revealed more toxicity [24].

It would be important to carry out more studies for the identification of toxic effects of iron oxide nanoparticles on various tissues with different concentrations, nanoparticle sizes, and routes of administrations.

Conclusion

The spherical shaped iron oxide nanoparticles with an average diameter of 50 nm are relatively safer in the dosage of 125 mg/kg, 250 mg/kg and 500 mg/kg body weight when administered orally.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Nanoparticles: Properties, applications and toxicities. Ibrahim Khan, Khalid Saeed, Idrees Khan. Arabian Journal of Chemistry. 2019; 12 (7): 908-31. DOI:10.1016/j.arabjc.2017.05.011.

2. Khandel P, Shahi SK. Microbes mediated synthesis of metal nanoparticles: current status and future prospects. International Journal of Nanomaterials and Biostructures. 2016; 6(1): 1-24.

3. Ali A, Zafar H, Zia M, Ul Haq I, Phull AR, Ali JS, et al. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnol Sci Appl. 2016;9:49-67. DOI: 10.2147/NSA.S99986.

4. Farooqui AA , Iftikhar A , Waqas M , Chatha G , Tayyab H. Iron Deficiency Anemia in pregnant females coming for their 1st antenatal workup - A Cross-Sectional Study. Pakistan Journal of Medical and Health Sciences. 2019; 13(2):449-50.

5. Qazi RA, Wagan F, Taqi T, Hashmi IQJ, Hashmi KK, Hashmi AR , et al. Prevalence of Anemia in pregnancy at District Shaheed Benazir Abad, Sindh. Pakistan Journal of Medical and Health Sciences. 2018;12(3):1114-16.

6. Ashfaq M, Jabeen S, Hanif A. Frequency of Iron Deficiency Anemia in Nulliparous Pregnant Females during Last Trimester. Pakistan Journal of Medical and Health Sciences.2018;12(1): 379-81.

7. Afridi IUK , Afridi H , Riaz B, Sheikh GA. Frequency of Iron Deficiency Anemia in Children: Cross sectional survey of outpatients at Akhtar Saeed Trust Teaching Hospital, Lahore from 2016-17 . Pakistan Journal of Medical and Health Sciences.2017;11(4):1365-8.

8. Hashem F, Nasr M, Ahmed Y. Preparation and evaluation of iron oxide nanoparticles for treatment of iron deficiency anemia. International Journal of Pharmacy and Pharmaceutical Sciences. 2018; 10(1): 142-6. DOI:10.22159/ijpps.2018v10i1.22686.

9. Karaagac O, Kockar H. Effect of synthesis parameters on the properties of superparamagnetic iron oxide nanoparticles. J Supercond Nov Magn. 2012; 25(8): 2777-81.

10. Fatima M, Zaidi NU, Amraiz D, Afzal F. In Vitro Antiviral Activity of Cinnamomum cassia and Its Nanoparticles Against H7N3 Influenza A Virus. J Microbiol Biotechnol. 2016;26(1):151-9. DOI: 10.4014/jmb.1508.08024.

11. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine. 2017;12:1227–1249. DOI:10.2147/IJN.S121956.

12. Kędziora A, Speruda M, Krzyżewska E, Rybka J, Łukowiak A, Bugla-Płoskońska G. Similarities and Differences between Silver Ions and Silver in Nanoforms as Antibacterial Agents. Int J Mol Sci. 2018;19(2):444. DOI:10.3390/ijms19020444

13. Baetke SC, Lammers T, Kiessling F. Applications of nanoparticles for diagnosis and therapy of cancer. Br J Radiol. 2015; 88(1054):20150207. DOI:10.1259/bjr.20150207

14. Ebrahimi E, Akbarzadeh A, Abbasi E, Khandaghi AA, Abasalizadeh F, Davaran S. Novel drug delivery system based on doxorubicin-encapsulated magnetic nanoparticles modified with PLGA-PEG 1000 copolymer. Artif Cells Nanomed Biotechnol. 2016; 44:290–7.
15. Nigam S, Bahadur D. Doxorubicin-loaded dendritic-Fe3O4 supramolecular nanoparticles for magnetic drug targeting and tumor regression in spheroid murine melanoma model. Nanomedicine. 2018;14(3): 759–68.
16. Yao Y, Zang Y, Qu J, Tang M, Zhang T. The Toxicity of Metallic Nanoparticles on Liver: The Subcellular Damages, Mechanisms, And Outcomes. Int J Nanomedicine. 2019;14:8787–804. DOI:10.2147/IJN.S212907
17. Du B, Yu M, Zheng J. Transport and interactions of nanoparticles in the kidneys. Nat Rev Mater.2018; 3: 358–74. DOI: 10.1038/s41578-018-0038-3
18. Song X, Gu X, Sun H, Fu C, Zhang Y, Dong P. Biomimetic modification and in vivo safety assessment of superparamagnetic iron oxide nanoparticles. J Nanosci Nanotechnol. 2016; 16: 4100–7.
19. Sohrabijam Z, Saeidifar M, Zamanian A. Enhancement of magnetofection efficiency using chitosan coated superparamagnetic iron oxide nanoparticles and calf thymus DNA. Colloids Surf B Biointerfaces. 2017;152:169-75. DOI:10.1016/j.colsurfb.2017.01.028.
20. Bellusci M, La Barbera A, Padella F, Mancuso M, Pasquo A, Grollino MG, et al. Biodistribution and acute toxicity of a nanofluid containing manganese iron oxide nanoparticles produced by a mechanochemical process. Int J Nanomed. 2014;9: 1919–29.
21. Sun B, Liu R, Ye N, Xiao ZD. Comprehensive evaluation of microRNA expression profiling reveals the neural signaling specific cytotoxicity of superparamagnetic iron oxide nanoparticles (SPIONs) through N-methyl-D-aspartate receptor. PLoS ONE. 2015; 10: e0121671.
22. Sadeghi L, Tanwir F, Yousefi Babadi V. In vitro toxicity of iron oxide nanoparticle: Oxidative damages on Hep G2 cells. Exp Toxicol Pathol. 2015; 67: 197–203.
23. Rajiv S, Jerobin J, Saranya V, Nainawat M, Sharma A, Makwana P, et al. Comparative cytotoxicity and genotoxicity of cobalt (II, III) oxide, iron (III) oxide, silicon dioxide, and aluminum oxide nanoparticles on human lymphocytes in vitro. Hum Exp Toxicol. 2016; 35:170–83.
24. Feng Q, Liu Y, Huang J, Chen K, Huang J, Xiao K. Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. Sci Rep. 2018; 8(1): 2082. DOI:10.1038/s41598-018-19628-z.

How to cite this article:
Mubin Mustafa Kiyani, Syed Ali Imran Bokhari, Asmara Syed, Hamza Rehman, Ekramy Elmorsy, Syed Sajid Hussain Shah. A histological study for the evaluation of potential harmful effects of orally ingested iron oxide nanoparticles in mice. Ann Clin Anal Med 2021;12(3):238-241